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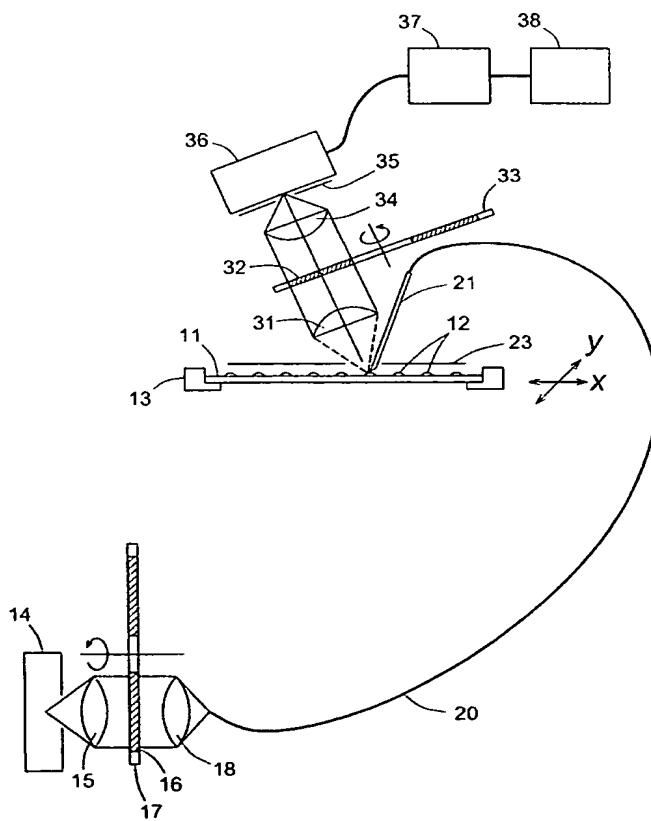
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(54) Title: BIOCHEMICAL ASSAY DETECTION IN A LIQUID RECEPTACLE USING A FIBER OPTIC EXCITER



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(57) Abstract: Biochemical assays that are performed in cuvettes and in the wells of multi-well plates and that utilize excitation and light emission as labels for detection are enhanced by an illumination and detection system that supplies excitation light through an optical fiber that transmits excitation light from an excitation light source to the cuvette or well. Emission light produced by the excitation is then collected by a collimating lens and converted to a signal that is compiled by conventional software for analysis. The optical fiber and collimating lens can either be on the same side of the receptacle (generally the open side) or on opposite sides, i.e., one above and the other below. When the optical fiber and the collimating lens are both on the open side of the receptacle, they are arranged such that the direction of travel of the excitation light and the direction along which the emission light is collected are not coaxial, and preferably both are at an acute angle to the axis normal to the mouth of the receptacle. Illumination systems are also disclosed in which a ultraviolet, visible, or near-infrared light source is optically coupled to an optical fiber.

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BIOCHEMICAL ASSAY DETECTION IN A LIQUID RECEPTACLE USING A FIBER OPTIC EXCITER

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

[0001] This invention resides in the field of biochemical assays performed in receptacles such as cuvettes or the wells of multi-well plates such as MICROTITER® plates, and particularly in obtaining the assay results by optical excitation of the receptacle contents and detection of the emissions resulting from the excitation.

10 2. Description of the Prior Art

[0002] Fluorescence and other optical signals are widely used in biochemical assays, particularly as a label in distinguishing test species that have demonstrated a sought-after property or characteristic in the assay from those that have not. Assays utilizing optical signals are frequently performed in liquid or fluid media retained in a sample receptacle, and 15 optical measurements are performed either on species suspended in the liquid media or on species adhering to the walls of the receptacle, such as species immunologically bound to the walls of microplate wells or cells plated to the bottoms of cuvettes. Instrumentation in current use for measuring fluorescence include a light source and a lens system for focusing a beam into the sample receptacle, and an optical system for collecting and processing the 20 emission light that results from the excitation. The two frequently interfere with each other, however, resulting in a loss of assay sensitivity.

[0003] In one type of excitation and emission detection system, a pierced mirror, which is either flat or elliptical, is used for both directing the excitation light to the sample receptacle and collecting the emission light that is produced. Pierced-mirror systems have limited 25 sensitivity, however, since the need to optimize the collection of the weaker emission light requires compromises that result in loss of much of the excitation light. As a result, these

devices suffer from a reduced intensity due to restricted aperture considerations and to the misdirection of a portion of the excitation light.

5 [0004] Other systems use a dichroic mirror to separate the excitation and emission light which are otherwise along a common path. The use of a dichroic mirror simplifies the optical path and instrument layout, but efficient separation of the emission light from the excitation light requires an expensive optical filter and a reduction in the signal light. Dichroic mirror systems are principally used in microscopy where light is abundant, rather than in systems where trace amounts of fluorophore are detected with low levels of emission light.

SUMMARY OF THE INVENTION

10 [0005] The present invention resides in illumination and detection systems for biochemical assays performed in small liquid receptacles such as cuvettes, small test tubes, or the wells of a multi-well plate. The receptacle is illuminated with a divergent beam of excitation light from an optical fiber, and emission light generated in the receptacle as a result of the excitation is collected, preferably without the use of an optical fiber, by a collimating lens
15 and converted by a detector to a signal that can be quantified, recorded, and otherwise processed by conventional instrumentation components. When the receptacle is one well of a multi-well plate such as a MICROTITER® plate, further signals are obtained by rastering either the plate or the optical system until detection has been performed on all wells of the plate. This system is applicable to any receptacle in which an assay is performed that utilizes
20 a label that emits a signal upon optical excitation. Preferred labels are fluorophores and fluorescent emissions, but the invention extends as well to phosphors and other types of optically excitable labels known to those familiar with biochemical assays.

[0006] In preferred embodiments of the invention, the optical system is arranged such that the direction of travel of the excitation light and the direction along which the emission light
25 is collected are two different directions, i.e., the two paths do not have a common axis. The optical fiber is configured to illuminate the test area either by epi-illumination (i.e., directing excitation light to the receptacle contents through the open mouth of the receptacle and collecting emission light through the open mouth as well) or by trans-illumination (using a light-transmissive receptacle or one with a light-transmissive floor, and either directing
30 excitation light to the receptacle from above and collecting emission light from below through the light-transmissive floor, or directing excitation light from below through the light-transmissive floor and collecting emission light from above). In multi-well plates,

illumination and emission detection can likewise occur at opposite sides of the plate, either by illuminating from below (the underside of the plate) and collecting emission from above (through the mouth of a well), or by illuminating from above and collecting emission from below. In systems utilizing epi-illumination, the optical fiber and the direction along which
5 the emission light is collected are not coaxial, instead forming an angle to each other. In trans-illumination as well, the optical fiber and the optical path used for collection of the emission light are in a non-coaxial arrangement. Preferred optical fibers for use in this invention are those that produce a divergent angle sufficient to illuminate the entire receptacle including all of the test materials contained in the receptacle, and to illuminate
10 only that receptacle, i.e., without illuminating materials in neighboring receptacles.

[0007] Preferred excitation light sources are those supplying ultraviolet, visible, or near-infrared light, optically coupled with the optical fiber so that substantially all of the light from the light source enters the fiber for transmission to the receptacle interior. This will result in substantially no loss of intensity between the light source and the receptacle. The optical
15 fiber itself may be a simple fiber or one that includes such elements as a collimator or optical filter, or a collimator-filter-collimator assembly, to process the light in various ways that will enhance its use for particular applications and assays.

[0008] Systems in accordance with this invention offer numerous advantages over the prior art. By separating the excitation and emission light paths, systems in accordance with this
20 invention limit the excitation and emission optics to a single function each, thereby permitting individual optimization of these two optical systems. In epi-illumination systems, little of the excitation light is detected in the emission light path and a maximal signal-to-noise ratio is achieved, particularly when the illumination fiber and the emission collection optics are at different angles relative to the normal axis. When the system is used on multi-
25 well plates, only a single well is illuminated by the optical fiber and emission is collected from that well individually, thereby eliminating crosstalk and maximizing the signal of that well. This leads to maximal signal collection and superior performance for any given level of detection and sensitivity. Furthermore, excitation systems of this invention require no additional lenses or other optical elements between the excitation fiber and the assay
30 receptacle, and can thus avoid the losses in light intensity that are often caused by these additional elements. In aspects of this invention that are directed to an optical fiber optically coupled directly to an LED or SLD light source, the coupled product is inexpensive, durable, and compact, and delivers bright light while generating minimal heat. This simplified yet highly efficient design presents advantages for packaging, cost and size reduction.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 is a diagram of an excitation and detection system for a multi-well plate utilizing the features of the present invention.

5 [0010] FIG. 2 is a diagram of an excitation system for use in conjunction with the excitation and detection system of FIG. 1, using an LED or SLD light source optically coupled to the fiber and an optical filter on the output end of the fiber.

[0011] FIG. 3 is a diagram of a second excitation system for use in conjunction with the excitation and detection system of FIG. 1, also using an LED or SLD light source optically coupled to the fiber and an optical filter on the output end of the fiber.

10 **DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS**

[0012] Receptacles in which bioassay detection can be performed by use of the present invention include cuvettes, small test tubes, wells of multi-well plates, and any variety of vessels capable of containing the components used in performing biochemical assays.

15 Examples of multiwell plates are MICROTITER® plates, as well as plates bearing different designations but generally containing either a row of wells or a two-dimensional rectangular array of wells. The most commonly used multi-well plates are those with a 96-well (12 × 8) array. Others have 6-well, 12-well, 48-well, 384-well, and 1536-well arrays. Typical well diameters or widths are from about 4 mm to about 40 mm, and preferably from about 4 mm to about 11 mm. The emissions to be generated and detected in the practice of this invention can arise from liquid retained in the receptacle, from substances suspended in the liquid, or from substances adhering to the receptacle walls.

20

[0013] The excitation system of this invention may contain any of a variety of different types of light sources. Examples are broadband light sources such as xenon flash lamps, 25 quartz halogen lamps, light-emitting diodes (LEDs), vertical cavity surface-emitting lasers (VCSELs), superluminescent diodes (SLDs), and narrowband sources such as single or multiple discrete wavelength lasers. In preferred systems, the light source is optically coupled to the optical fiber. For point sources of light or sources such as solid state sources that are nearly point sources, optical coupling may be achieved by proximity coupling.

30 Otherwise, a coupling lens or lens system that will transmit substantially all of the light from the light source to the fiber can be used. The system can also include an optical excitation filter, a monochromator, or a tunable acousto-optic filter. The optical fiber transmits light

from the light source to the receptacle to excite either a single label or multiple labels that are present in the receptacle. Emission light resulting from the excitation is collected by a collimating lens system which directs the light to a detector, optionally first passing through an optical emission filter, a monochromator, or a tunable acousto-optic filter. Examples of 5 suitable detectors are photomultiplier tubes, microchannel plates, silicon PIN diodes, avalanche photodiodes (APDs), CCD detectors, and CMOS detectors.

[0014] The optical fiber is either straight or tapered along its length. The choice of fiber may vary with the particular type of receptacle to which the light is directed. A multi-mode fiber with cladding and outer buffer coating is preferred, particularly one with a divergence 10 angle suitable for filling the interior of the receptacle. Such fibers are commercially available from various suppliers well known to those in the industry. One such supplier is 3M Company, St. Paul, Minnesota, USA, and another is Polymicro Technologies, LLC, Phoenix, Arizona, USA.

[0015] The optical fiber can have either a standard tip or a shaped tip on either or both of 15 its ends, or a combination of both. In certain embodiments of the invention, the delivery end preferably has a shaped tip such as a concentrator cone tip. The tip may also have an integral lens. The divergence angle at the distal tip is preferably within the range of about 10 degrees to about 60 degrees, most preferably from about 10 degrees to about 20 degrees. In terms of the numerical aperture (the sine of the divergence angle θ), the preferred range is from about 20 0.17 to about 0.94, and most preferably from about 0.17 to about 0.34. For MICROTITER® plates, a presently preferred numerical aperture is 0.22 (a divergence angle of 12.7 degrees). Optical fibers with shaped tips as described in this paragraph are available from Polymicro Technologies referenced above.

[0016] The tip of the optical fiber is preferably placed very close to the mouth of the 25 receptacle, particularly in the case of receptacles that are wells in multi-well plates where illumination of neighboring wells is sought to be avoided. Preferably, the fiber tip is placed within 1 mm to 10 mm of the mouth of the well.

[0017] The vessel in which the receptacle is formed can be opaque or light-transmissive, and light-transmissive vessels can be translucent or transparent. When a light-transmissive 30 vessel is used, illumination of the receptacle interior with excitation light can be achieved from the underside of the vessel, i.e., through the light-transmissive bottom of the vessel ("trans-illumination"). The angle of incidence of the excitation light is defined relative to the axis normal to the mouth of the receptacle, and this angle is not critical and may vary. Likewise, the direction along which the collimating lens collects the emission light can vary.

In systems where trans-illumination is used, it is preferred that the emission light be collected along an acute angle relative to the axis. Preferred such angles are at least about 5° and most preferably from about 5° to about 15°. In systems involving epi-illumination, the optical fiber and the direction along which the emission light is collected are non-coaxial, and it is preferred that the fiber and the emission collection direction each form angles (relative to the axis) of from about 5° to about 60°, and more preferably from about 5° to about 25°. When the receptacle is a multi-well plate, a presently preferred angle for the optical fiber (relative to the axis) in epi-illumination systems is 10°, with the emission collection path at 15° relative to the fiber, while in trans-illumination systems a presently preferred angle for the optical fiber is 5-10° (relative to the axis), with the emission collection path along the axis itself.

[0018] Of the various types of light sources that can be used in the practice of this invention, broadband LEDs are preferred. This and other types of light source can also include a phosphor or other broadband conversion element upstream of the coupling to the fiber. The conversion element can be a coating on the light source, or it can be incorporated into the plastic packaging of the light source or in a gel or other discrete closed package. As a further alternative, the conversion element can be intagliated into the end of the fiber itself.

[0019] The optical coupling between the light source and the optical fiber can be achieved by a focusing lens or lens system located between the light source and the optical fiber, or by direct coupling of the optical fiber to the light source. Examples of focusing lens systems are a ball lens, a pair of microscope objectives, and a condenser pair of plano convex lenses.

Proximity coupling, i.e., direct coupling of the fiber to the light source, is preferred. LEDs and SLD's, which are readily available from commercial suppliers, can be readily modified by removing the lens system supplied by the manufacturer and placing the flat fiber end very close to, and preferably in direct secured contact with, the glowing LED itself. An optically clear cement with low autofluorescence can be used. Examples of such a cement are Norland Optical Cement NOA 73 and NOA 61, Norland Products, Inc., Cranbury, New Jersey, USA. Alternatives to cements are gels or oils that are optically clear. To stabilize the coupling, the LED or SLD and the fiber end can be encased in a metal tube, a straight tip (ST) connector, or any other packaging, with one end of the connector joined to the LED or SLD and the other to the optical fiber.

[0020] While the novelty-defining concepts and features of the invention can be implemented in many different configurations and arrangements, a convenient way to achieve

an understanding of these features is to study individual systems within the scope of the invention. Such system are depicted in the Figures.

[0021] The system shown in FIG. 1 is arranged for signal generation and detection in a multi-well plate 11 such as a MICROTITER® plate, each well 12 of which is partially filled with liquid components of a biochemical assay. The contents of each well have been treated with any of various fluorochrome dyes or probes or any other labels that when irradiated with an excitation light beam respond by producing emission light. The well diameter can be less than 4 mm or greater than 36 mm in diameter, depending on the number of wells in the plate. The plate 11 is held by a holding fixture (not shown) which moves or rasters the plate in the x and y directions within a plane that is transverse to the direction of the optical paths through which excitation and emission collection are performed. This rastering movement enables the system to capture signals sequentially from the entire array of wells. As an alternative arrangement, the plate can be held stationary and the optics made movable in the x and y directions across the sample surface.

[0022] An excitation light source 14, which may be a broadband source such as for example a xenon lamp, a quartz halogen lamp, an LED, an SLD, or a narrow band source such as for example a single or multiple discrete wavelength laser, illuminates a collimating lens 15. Alternatively, two or more discrete lasers can be used simultaneously with a single fiber. The collimated light emerging from the collimating lens 15 passes through an optical excitation filter 16 on a multi-position filter wheel 17. The filter wheel permits the selection of particular excitation wavelengths from a variety of wavelengths, and its rotation and position can be controlled by software appropriately adapted to particular experimental protocols. As alternatives to the optical excitation filter, a monochromator or a tunable acousto-optic filter may be used. A second lens 18 focuses the light and couples it into either of two optical fibers 19, 20. The fibers terminate in fiber holders 21, 22, respectively, the output end of each fiber holder being positioned in close proximity to a well 12 on the plate 11. These two optical fibers offer alternative means of providing excitation light to the well, one fiber 21 delivering the excitation light to the top side of the plate through the open mouth 23 of the well for epi-illumination and the other fiber 22 delivering the excitation light to the underside 24 of the plate for trans-illumination of the well through the plate itself.

[0023] In the system shown in FIG. 1, no additional lenses or optics are located between the fiber holders 21, 22 and the well plate 11. Light from the fiber tip 23 produces a cone-shaped beam of excitation light which fills the well 12. The divergence of the light cone can also be controlled or modified by either specialized cutting, polishing, or angling of the fiber

end. Optionally, a mask 24 can be positioned above the sample to further assure that the well of interest does not receive any stray excitation or emission light from neighboring wells or from dust or other contaminants.

[0024] The emission light that the well 12 emits upon excitation is collected by a collimating lens 31, and the collimated emission light passes through an optical emission filter 32 on a multi-position filter wheel 33. Alternatively, a monochromator or tunable acousto-optic filter can be used in place of the optical emission filter 32. A second lens 34 then focuses the collimated light through an aperture 35 to control stray light and onto a light detector 36. Examples of suitable light detectors are photomultiplier tubes, silicon PIN diodes, avalanche photodiodes (APDs), CCD detectors, and CMOS detectors. The detector 36 registers the emission light intensity and sends an output signal to a recorder or to processing and control electronics 37.

[0025] These systems and other systems within the scope of this invention are readily adaptable to achieve signal generation and processing by Time-Resolved Fluorescence. This is accomplished for example by using a flashlamp, an LED, or an SLD as the light source, imposing a controlled delay time between a flash of the light source and the signal collection, and allowing for programmable variable signal collection integration time. The only modifications needed to achieve this are modifications of the software and electronics, and such modifications will be readily apparent to those skilled in the art of Time-Resolved Fluorescence.

[0026] Although not shown in the drawings, the system can include two or more optical excitation fibers arranged either in a linear array or an x-y (two-dimensional) array rather than in a single fiber. Each individual excitation fiber is associated with a separate collection channel, and the entire fiber array can be moved across the well plate, or the plate moved relative to the fiber array, and in either case signals are obtained from all wells of the plate in a shorter span of time than a single fiber.

[0027] Examples of illumination systems in accordance with this invention utilizing an LED or SLD light source are shown in FIGS. 2 and 3.

[0028] The system of FIG. 2 includes an LED or SLD source 41, preferably a white light LED or SLD, directly coupled to the flat end of an optical fiber 43 through an optical cement such as those described above. The coupling is surrounded by an epoxy potting compound 42 or a ring or tube. Light emerging from the LED or SLD is efficiently collected by the optical fiber 43 and transmitted to a fiber-optic device 44, which consists of a first fiber collimator for the light emerging from the LED or SLD, an optical bandpass filter, and a

second fiber collimator for the light emerging from the optical bandpass filter. The filter can either be a single optical bandpass filter or multiple filters mounted on a wheel or slide, allowing the user, either manually or by automated means, to select a particular filter and thereby excite a specific fluorophore. The light emerging from the second fiber collimator
5 returns to the fiber for delivery from the fiber tip 45 to a test region 46 on a multi-well plate.

[0029] The system of FIG. 3 has the same components as the system of FIG. 2 except that the sealant and packaging 42 of FIG. 1 are replaced by a compact lens or lens system 52.

[0030] The foregoing descriptions are offered primarily for purposes of illustration. Further modifications, variations and substitutions that still fall within the spirit and scope of
10 the invention will be readily apparent to those skilled in the art.

WHAT IS CLAIMED IS:

1 1. Apparatus for generating and detecting an optical signal from the
2 contents of a liquid receptacle resulting from a bioassay performed therein, said apparatus
3 comprising:

4 an excitation light source,
5 an optical fiber arranged to transmit excitation light from said excitation light
6 source to said receptacle with a divergent beam, and
7 a collimating lens arranged to collect emission light from said receptacle; and
8 detection means for receiving emission light collected by said collimating lens
9 and for generating a signal representative of said emission light thus collected.

1 2. Apparatus in accordance with claim 1 in which said receptacle has an
2 open mouth, said optical fiber is arranged to direct said excitation light through said open
3 mouth, and said collimating lens is arranged to collect said emission light through said open
4 mouth along an emission collection path that is not coaxial with said optical fiber.

1 3. Apparatus in accordance with claim 2 in which said open mouth
2 defines an axis normal thereto, and said optical fiber and said emission collection path form
3 an angle of from about 5° to about 25° relative to said axis.

1 4. Apparatus in accordance with claim 1 in which said receptacle has a
2 light-transmissive floor and an open mouth opposite said light-transmissive floor, said optical
3 fiber is arranged to transmit light to the contents of said receptacle through one of said light-
4 transmissive floor and said open mouth, and said collimating lens is arranged to collect said
5 emission light through the other of said light-transmissive floor and said open mouth.

1 5. Apparatus in accordance with claim 1 in which said excitation light
2 source is a light-emitting diode and said optical fiber is secured to, and in direct contact with,
3 said diode, either by direct coupling or through a member selected from the group consisting
4 of an optically clear adhesive, an optically clear gel, an optically clear oil, and a lens system
5 directing light from said diode into said optical fiber.

1 6. Apparatus in accordance with claim 1 in which said liquid receptacle is
2 a well of a multi-well plate, and said apparatus further comprises means for rastering either
3 said multi-well plate relative to said optical fiber and said collimating lens or said optical

4 fiber and said collimating lens relative to said multi-well plate until light from said excitation
5 light source has illuminated, and emission light has been collected from, all wells of said
6 multi-well plate.

1 **7.** A method for generating and detecting an optical signal from the
2 contents of a liquid receptacle resulting from a bioassay performed in said receptacle, said
3 method comprising:

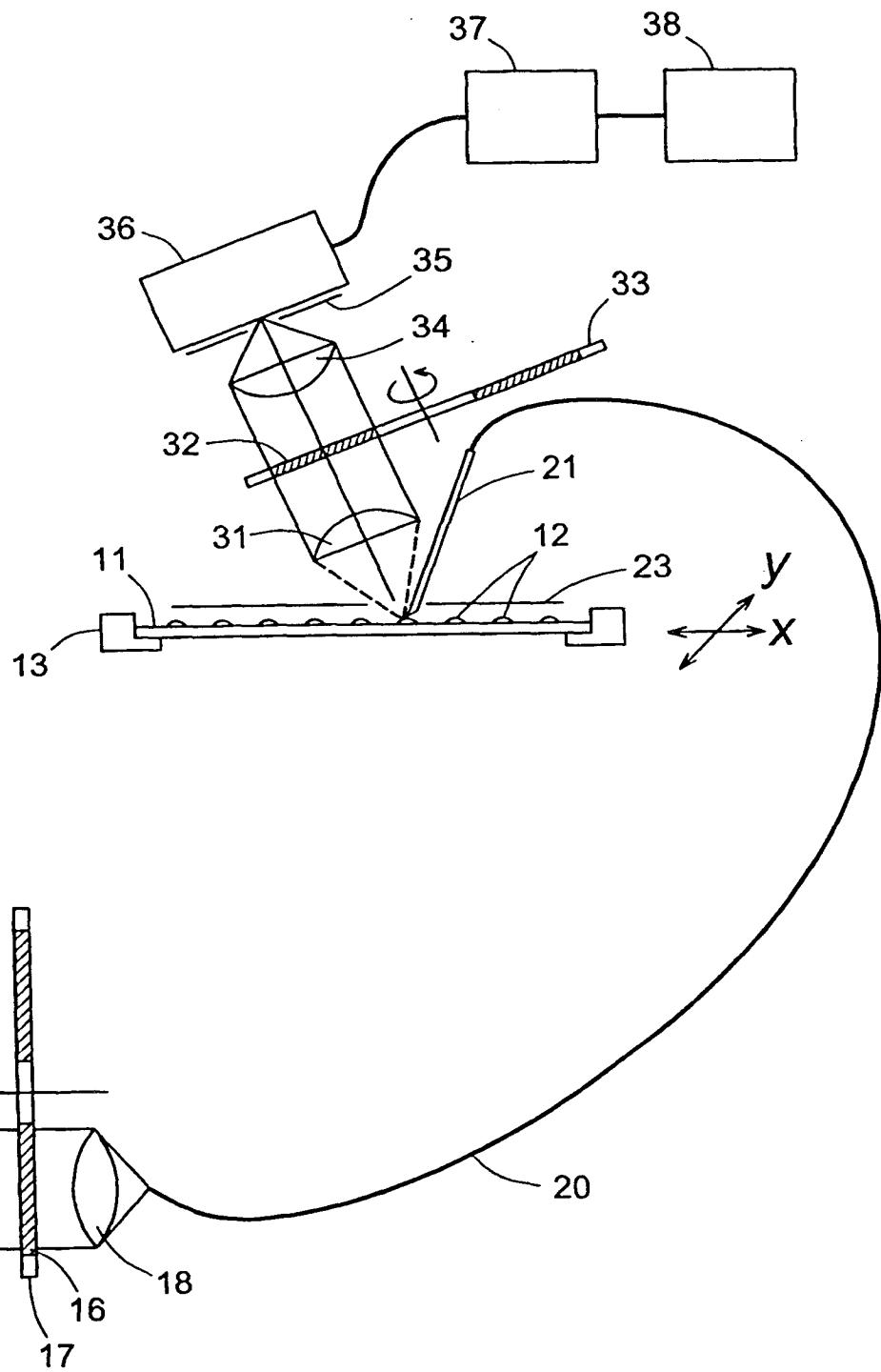
4 (a) illuminating the contents of said receptacle with light from an excitation
5 light source through an optical fiber directing a divergent beam at said receptacle; and
6 (b) collecting emission light from said receptacle by a collimating lens and
7 directing emission light thus collected to a detector.

1 **8.** A method in accordance with claim 7 in which said receptacle has an
2 open mouth and said method comprises illuminating the contents of said receptacle through
3 said open mouth and collecting said emission light from said receptacle through said open
4 mouth.

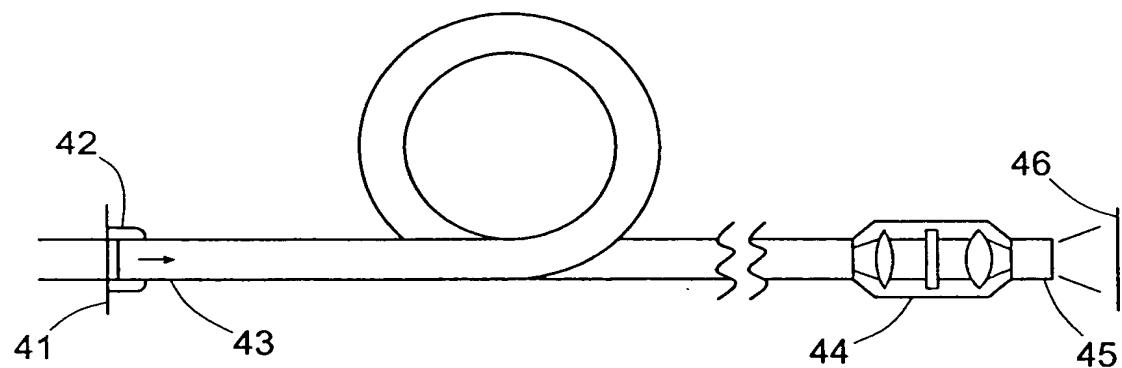
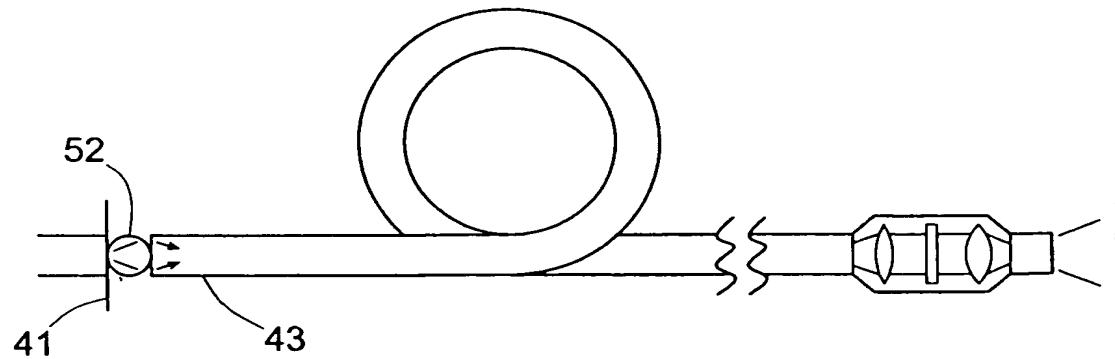
1 **9.** A method in accordance with claim 7 in which said receptacle has a
2 light-transmissive floor and an open mouth opposite said light-transmissive floor, step (a)
3 comprises directing said divergent beam through one of said light-transmissive floor and said
4 open mouth, and step (b) comprises collecting said emission light through the other of said
5 light-transmissive floor and said open mouth.

1 **10.** A method for generating and detecting optical signals from a plurality
2 of biochemical assays performed in individual wells of a multi-well plate, said method
3 comprising:

4 (a) illuminating the contents of a single well with light from an excitation
5 light source through an optical fiber directing a divergent beam at said well;
6 (b) collecting emission light from said well by a collimating lens and
7 directing emission light thus collected to a detector; and
8 (c) rastering either said multi-well plate relative to said optical fiber and said
9 collimating lens or said optical and said collimating lens relative to said multi-well
10 plate until light from said excitation light source has illuminated, and emission light
11 has been collected from, all wells of said multi-well plate.

Fig. 1

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Fig. 2*Fig. 3*

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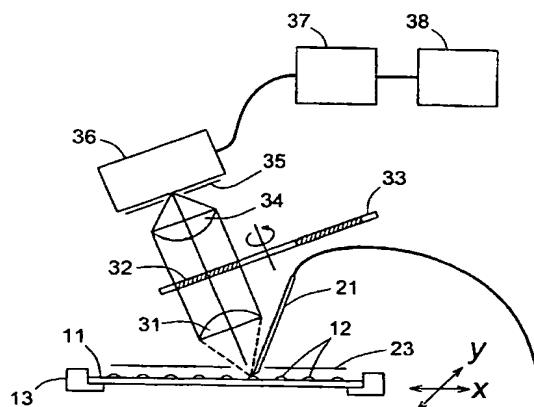
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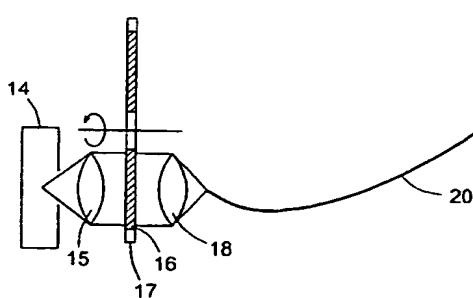
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[Continued on next page]

(54) Title: BIOCHEMICAL ASSAY DETECTION IN A LIQUID RECEPTACLE USING A FIBER OPTIC EXCITER



WO 03/027741 A3



(57) Abstract: Biochemical assays that are performed in multi-well plates (11) and that utilize a system that supplies excitation light through an optical fiber (21, 22) that transmits light from an excitation light source (14) to the well (12). Emission light produced by the excitation is then collected by a collimating lens (31) and converted to a signal that is compiled for analysis. The optical fiber (21, 22) and collimating lens (31) can either be on the same side of the receptacle or on opposite sides, i.e., one above the other below. When the optical fiber and the collimating lens are both on the open side of the receptacle, they are arranged such that the direction of travel of the excitation light and the direction along which the emission light is collected are not coaxial, and preferably both are at an acute angle to the axis normal to the mouth of the receptacle.



ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/30301

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G02B 6/32
US CL : 385/33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 385/33, 31, 15; 356/342

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPAT, US-PGPUB, EPO, JPO, Derwent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,016,195 A (PETERS) 18 January 2000 (18.01.2000), Figs. 3, 4, 6.	1-5, 7-9
A	US 5,116,759 A (KLAINER et al) 26 May 1992 (26.05.1992), see the entire document.	1-10

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"P" document published prior to the international filing date but later than the priority date claimed		

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